EXHIBIT O

TO DECLARATION OF SCOTT D. TANNER, PHD.

U.S. Patent Application Ser. No. 10/614,115

REVIEW

Element and molecular mass spectrometry—an emerging analytical dream team in the life sciences

Mathias Windt and Wolf D. Lehmann*

Central Spectroscopy, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. E-mail: wolf.lehmann@dkfz.de; Fax: 0049 - 6221 - 424554; Tel: 0049 - 6221 - 424563

Received 7th August 2003, Accepted 27th October 2003
First published as an Advance Article on the web 10th December 2003

The recent literature on the combined applications of element (ICP, inductively coupled plasma) mass spectrometry and molecular (ESI, electrospray ionisation and MALDI, matrix assisted laser desorption ionisation) mass spectrometry in the life sciences is reviewed. Emphasis is put on investigations where both techniques have been used in a synergistic way with the aim of an analytical result with molecular specificity. Multi-element specificity and quantification are the key features of element MS, whereas molecular weight determination and structural information are those contributed by ESI and MALDI MS. The analytical applications cover the study of selenoproteins, phosphoproteins, metalloproteins, unmodified proteins, metallothioneins, phytochelatins, nucleic acids, arsenosugars, thyroid hormones, cobalamines, drugs and drug metabolites. Supported by on-line or off-line coupling to chromatographic or electrophoretic separation methods, the combined application of element and molecular mass spectrometry in all these studies has created the basis for new structural and/or quantitative insights, demonstrating the analytical excellence of this approach.

Introduction

Inductively coupled plasma mass spectrometry (ICP-MS), also termed element MS, is an established tool for quantitative element trace analysis and for speciation of elements in biological samples. In the last decade, innovative applications of ICP-MS coupled to chromatography are accumulating in life sciences projects where this technique is applied in combination with molecular MS techniques (ESI and MALDI-MS). This emerging combination of element and molecular mass spectrometry opens new possibilities for the spotting, identification and quantification of biomolecules with previously unknown structure. Classically, this field is often referred to as bioinorganic analysis. Earlier reviews on this area are focused on methodological aspects, such as the coupling of the various chromatographic methods to ICP-MS,[1–4] and the bulk of applications is found in the area of metal speciation.

In recent years, an innovative trend can be observed towards joint applications of element and molecular mass spectrometry for specific detection and molecular characterization of complex biomolecules containing an ICP-detectable element (ICP-tag). Another innovative feature in many of these studies is the application of ICP-MS for the analysis of metalloid or non-metal elements. The main constituents of organic molecules—C, H, N and O—can be considered as elements which are outside the scope of ICP-MS (hydrogen is not detectable and analysis of C, N, O requires elaborate efforts, so far preventing a routine application). However, all other elements of the biosphere can be detected by ICP-MS and can therefore be denominated as ICP-tag. In a number of biomolecules one or more of these ICP-tags are naturally present. If desired, they can also be introduced intentionally by a suitable derivatization. This concept opens a spectrum of new analytical options and strategies, since these ICP-tags comprise P, S, Se, Si, Cl, Br, I, As, and all metals. Bioanalytes must exhibit a certain thermodynamic and kinetic stability to be preserved in their native compositional and coordinative structure during their chromatographic separation and their molecular detection by MS. Therefore, this approach is most successful when applied to molecules, which contain their ICP-tag bound in a covalent or covalent-like form.

In contrast, metal ions weakly coordinated to organic ligands so far are difficult to analyze by this methodology,[5] and if results are obtained, their biological impact has to be discussed with caution. In addition, ICP-sensitivity covers a broad range, *e.g.* P and S are detected with significantly lower sensitivity than are metals.

In spite of these somewhat limiting features, element and molecular mass spectrometry represent a truly supplementary team of techniques for exploratory analytical tasks in the life sciences. To underline this statement, this review gives reference to a collection of recent studies in the life sciences to which both techniques have provided significant input, finally leading to new structural or quantitative insights at the molecular level. According to this focus, ICP-MS studies without strong reference to individual molecular aspects were not considered, among these are for instance numerous studies centered on the speciation of metals in biological samples. This review is structured along compound classes, which comprise (i) amino acids, peptides and proteins, (ii) nucleotides and nucleic acids, (iii) arsenosugars, (iv) small biomolecules like hormones and vitamins and (v) drugs and drug metabolites.

1. Amino acids, peptides and proteins

Selenium-containing proteins and peptides. The recognition of selenium as an essential trace element in human nutrition has initiated studies on its chemical forms in food and food supplements enriched in selenium. The coupling of SEC-ICP-MS is widely used as a first step in these studies, sometimes followed by additional separation steps, since in general the purity of the selenium-containing fractions after SEC is not sufficient for mass spectrometric characterization. Using this approach Se-containing analytes were characterized in human milk,[6] garlic,[7,8] and in yeast extracts.[9–15] For instance, methylselenocysteine[7]

† Presented at the 2002 Winter Conference on Plasma Spectrochemistry, Scottsdale, AZ, USA, January 6–12, 2002.

DOI: 10.1039/b309482k

and γ-glutamyl-Se-methylselenocysteine[8] could be identified as the main selenium compound in garlic by ESI and ESI-MS/MS. In the recognition of Se-containing compounds in an ESI-MS survey spectrum, the characteristic Se isotope pattern is particularly helpful, consisting of ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se. For the interpretation of the corresponding ESI-MS/MS spectra, the Se isotopic heterogeneity is also of advantage, since MS/MS spectra of the single isotopomeric species can be generated. In the set of these spectra, the fragment ions containing Se show a shift related to the Se isotope present in the precursor ion, whereas all other fragment ions show up at a constant *m/z* value (see ref. 8). In yeast extracts it could be demonstrated that Se is present as seleno- methionine[9] and Se-adenosylselenohomocysteine.[9,10] In addition, the presence of the selenotrisulfides, selenodiglutathion and selenoglutathion-cysteinylglycine[11] could be demonstrated by nanoESI-MS/MS. These structures are similar to trisulfides described as occurring in cysteine-containing proteins. In the structural characterization of selenoproteins extracted from selenized yeast, and digested by trypsin, the use of MALDI-MS was found to be superior over ESI-MS for the recognition of the isotopic pattern of Se-containing species,[12,13] which then were structurally characterized by ESI-MS/MS. In this way, the random incorporation of Se-Met into two yeast proteins, SIP18 and HSP12, was demonstrated with high coverage of the selenium-containing peptides.[13] Whereas Se-Met is considered to be arbitrarily incorporated at any position normally occupied by Met, Se-Cys is specifically incorporated at particular positions of only a few selenoproteins. This process is directed by UGA mRNA codons within an environment changing their standard function as stop codon into a codon for Se-Cys incorporation.[16] In humans, three selenoproteins are known, selenoprotein P,[17] glutathion peroxidase,[18] and thyroglobin deiodinase.[19] Among these, the plasma protein selenoprotein P is that with the highest Se content of up to 12 Se-Cys units.[17] Using a combination of a heparin affinity column and a SEC column for two-dimensional chromatography in series, the main two selenocysteine containing proteins, selenoprotein P and glutathionperoxidase could be quantitatively determined by ICP-MS detection of selenium.[20] Selenoprotein P is believed to have redox functions, *e.g.* in the reduction of phospholipid hydroperoxides and possibly has an additional function in metal detoxification. The latter idea stems from various *in vitro* observations, including the stoichiometric interaction with Hg(II)[21] and the possibility to selectively enrich selenoprotein P with a Co(II) charged IMAC column.[22]

Due to the stable incorporation of Se in Se-Cys, selenoprotein P isolated from rat plasma could be characterized by a standard analytical proteomics procedure. Such a procedure is schematically outlined in Fig. 1.

In this analysis, three truncated isoforms of selenoprotein P were found besides the intact form.[23] Since all three forms ended with an amino acid preceding a Se-Cys residue (exactly before the 2nd, 3rd, and 7th residue) it was concluded that the UGA codons in this subgroup have a dual function, either encoding for the incorporation of Se-Cys or—as normal—encoding for chain termination. A somewhat lower chemical stability of Se-Cys residues compared to Cys residues under the standard analytical proteomics conditions was observed. For selenoprotein P, partial loss of SeH_2 resulting in dehydroalanine formation was observed under conditions which do not affect the stability of normal Cys residues.[24]

Unmodified proteins. The sulfur-containing amino acids cysteine and methionine together exhibit a natural abundance of about 4% of all amino acids, so that statistically every peptide/protein with more than 25 residues contains at least one atom of sulfur. Although there are some proteins which lack a C or M residue, the majority of proteins do contain

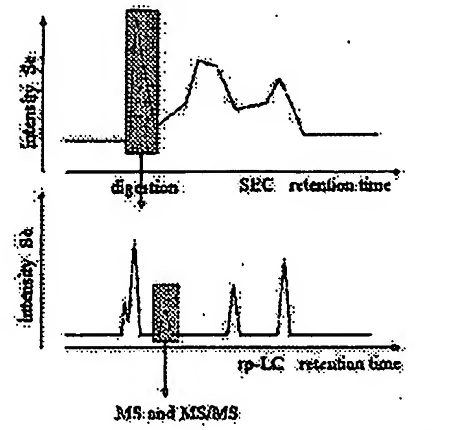

Fig. 1 Typical strategy for identification of Se in proteins: the Se-containing high molecular weight fraction is spotted by size-exclusion LC coupled to ICP-MS, this fraction is collected and digested by a protease. The digest is analyzed for Se-containing peptides by reversed-phase LC-ICP-MS. Se-containing fractions are collected and characterized by MALDI-MS for their molecular ion and by ESI-MS/MS for their structure (*e.g.* ref. 13).

sulfur. Thus, sulfur can be considered as a target element, by which unmodified proteins can be detected or quantified using ICP-MS. The latter approach has been used in coupling to capillary LC for quantification of insulin in a pharmaceutical preparation of human insulin.[25] The good agreement between the certified and the experimentally determined insulin concentration demonstrated the feasibility of this approach.

Phosphoproteins. Reversible phosphorylation at serine, threonine and tyrosine residues is a widespread covalent modification of proteins, by which many vital cellular functions are controlled.[26] It is estimated that about 1/3 of all cellular proteins are phosphorylated. Capillary LC-ICP-MS with phosphorus detection provides a specific access to phosphopeptides and phosphoproteins, since phosphorus covalently bound to peptides occurs mainly in this form. Separation from the ubiquitous inorganic phosphate is achieved by the use of reversed phase LC, since inorganic phosphate elutes non-retained with the solvent front. With a combination of ICP-MS with phosphorus detection and ESI-MS coupled to the same capillary LC, tryptic phosphopeptides can be recognized and identified.[27,28] This approach has also been successfully applied to the analysis of polo kinases with unknown phosphorylation sites.[29,30] A direct access to intact phosphoproteins isolated by gel electrophoresis is possible by the use of laser ablation ICP-MS with 31-P detection, which has been demonstrated by phosphoproteins present on blot membranes[31] or in gels.[32] A typical procedure is schematically outlined in Fig. 2.

The quantitative character of ICP-MS can be used for the determination of the degree of phosphorylation. When the phosphopeptide/protein contains at least one Cys or Met residue then the ratio of P over S can be determined. Based on the sensitivity factors for P and S, the experimental P/S ratio can be converted into the stoichiometric P/S ratio and thus into the degree of phosphorylation.[33,34] Spectral interferences on *m/z* 31 and *m/z* 32 were overcome by HR-ICP-MS using medium resolution (4000).[33] In the other approach, quadrupole ICP-MS was used to measure the phosphorylation degree of phosphoproteins in combination with a dynamic reaction cell.[34] For further reduction of polyatomic interferences, sulfur and phosphorus were detected as their oxides SO^+ and PO^+ generated by ion–molecule reactions in this case. The introduction of the collision and reaction cell technology has expanded the applicability of quadrupole instrumentation,

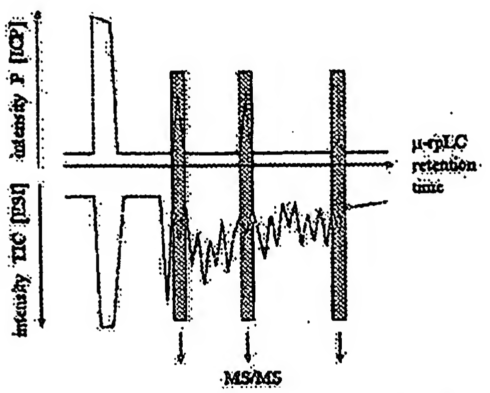

Fig. 2 Typical strategy for analysis of protein phosphorylation. A phosphoprotein is isolated and digested. The analysis of this digest is displayed. Reversed phase capillary LC coupled to ICP-MS with phosphorus detection and to ESI-MS is used. Phosphopeptides are recognized in the ^{31}P trace and characterized by ESI-MS and MS/MS. The latter technique can be performed either in the LC-MS mode, by nanoESI of the total digest or by nanoESI of isolated phosphopeptide fractions.

since it effects a significant reduction of polyatomic background interference and introduces a new dimension of methodological optimization[35,36] (selection of collision/reaction gases, of pressure and collision energy).

Metalloproteins. Proteins frequently contain one or more essential metal ions coordinated at their catalytic or functional center(s). In addition, metal ions involved in allosteric regulations of proteins may be bound to other sites. Metals are typically coordinated to histidine (N), cysteine (S) or carboxyl functions (O). Since these metal–protein interactions sometimes are labile under standard analytical conditions employed for chromatography or mass spectrometry, investigation of metal-containing proteins requires particular attention to assure that their native composition is retained. Size-exclusion chromatography (SEC) can be performed under physiological pH and ionic strength without sacrificing separation power. Moreover, several SEC buffers are compatible with direct coupling to ICP-MS. Thus, SEC-ICP-MS is an ideal first stage for element speciation in a biological sample. As an example, SEC-ICP-MS has been applied to the analysis of several metal ions in bovine liver standard reference material.[37] The major part of many metals (Mn, Fe, Cu, Zn, Cd) was found to be present in the low molecular weight region (13 kDa or less), which was ascribed to their binding to metallothionein (see below).

When applied to purified metalloproteins, the use of LC-ICP-MS alone (without molecular MS methods) can also lead to new, quantitative insights into protein structure. One aspect is the combination of metal and protein quantification, and the other is the quantification of more than one metal. In a study of two bacterial metalloproteases, an LC-ICP-MS system was set up, which provided sufficient sensitivity for detection of a single metal ion in a protein and chromatographic conditions could be established with sufficiently low metal background.[38] Zn could be identified as the natural metal in two bacterial membrane proteases at the molar ratio of metal/protein of 3 and 1, respectively. Using the same system, the absence of a metal cofactor could be demonstrated for another isolated bacterial protease.[38]

In a study of recombinant endothelial nitric oxide synthase, zinc and iron was quantified in the dimeric form of this enzyme. The presence of two atoms of heme iron and one atom of zinc per subunit was verified.[39] In a subsequent functional study LC-ICP-MS was used to prove that Zn is necessary for the dimerization of this enzyme but that it is not functionally important.[40]

Metallothioneines. Metallothioneins form a superfamily of small cysteine-rich proteins with strong complexing properties for metals such as Zn, Cu, Cd and various other metals. The 20 cysteine residues in metallothioneins can bind up to 7 bivalent metal ions in two spatially separated domains *via* thiolate-like coordination. Metallothioneins occur in many mammalian tissues (in particular liver, kidney and brain) and are considered to have essential functions in cellular transport of essential metals such as Zn and Cu as well as for binding and sequestering of toxic metals such as Cd. Since their discovery, numerous studies with hyphenated techniques have been performed[40,41] including the use of ICP-MS and ESI-MS as final methods of detecting the metal part, and ESI-MS for detection of the intact metallothionein–metal complexes or of the metallothionein ligands alone after disintegration of the complexes by post-chromatographic acidification.[42] The natural microheterogeneity of metallothionein isoforms and subisoforms is overlaid by the variable pattern of loading with different metals, a situation which makes molecular analysis of this group of metalloproteins a challenging task. As for the other examples given above, SEC-ICP-MS is often applied as the first step for the purification of metallothioneins, followed by some chromatographic technique with higher resolving power. HPLC-ICP-MS and HPLC-ESI-MS were successfully applied for the characterization of rat liver metallothionein (MT) species after cadmium bioinduction.[43,44] In human brain tissue, reduced levels of the MT-3 subform were found by a similar approach[45] and in this case the molecular identity was confirmed among other techniques by surface-enhanced laser desorption ionization-MS (SELDI-MS), a variant of the MALDI-technique. However, the most powerful chromatographic separations of metallothioneins so far have been achieved by direct coupling of CE-ICP-MS.[46,47] The ESI-MS analyses in these MT studies were usually performed by low-resolution instruments, which sometimes did not allow the unambiguous assignment of all MT–metal complexes due to the natural heterogeneity of MT ligands and the multiple combinations of incorporated metals. High resolution ESI-MS data are more informative for these analytical tasks. Thus, this situation will improve in parallel to the more frequent application of HR-ESI-MS equipment, which is currently observed.

Three studies using only molecular or element mass spectrometry, respectively, for the analysis of MTs will be mentioned here in addition, since they highlight a particular potential of the corresponding techniques.

(i) In an ESI-MS/MS study noncovalent complexes between a Zn-loaded metallothionein and a set of glutathione-related compounds were analyzed by ESI-MS/MS. A partial transfer of Zn from metallothionein to glutathione was only observed for oxidized (S-S-linked) derivatives,[48] a phenomenon which might be correlated with the function of metallothioneins in cellular Zn-transport.

(ii) A generic, analyte-independent isotope dilution analysis was applied in combination with CE-ICP-MS for metallothionein quantification: The sheath liquid supplied at in the CE-ICP interface is spiked with isotopically enriched metals and isotopically enriched sulfur (^{32}S or ^{33}S). Then continuous isotope ratio monitoring of the corresponding element isotopes is performed with ICP-MS, whereupon the various MT species are detected by alterations in this isotope ratio, since their elution introduces sulfur and metals with their natural isotope abundances.[49,50] In this way, quantification of selected chelated metals and of the MT ligands is performed separately, so that both the absolute amounts and the degree of saturation of individual MT subforms are accessible.

(iii) Molecular specificity is conferred to ICP-MS by labelling

antibodies with gold nanoparticles, which results in a stable-isotope immunoassay with a sensitivity comparable to the classical radio-immunoassay.[51,52]

Phytochelatins are small cysteine-containing metal-binding peptides, by which plants regulate their metal homeostasis. Biosynthetically, they are derived from glutathione and exhibit the core composition of $(\gamma\text{-Glu-Cys})_{n=2\text{-}7}$ with different end groups. Following Cd-induction, phytochelatin–Cd complexes were recognized by LC-ICP-MS[53] and the phytochelatin part of these complexes was characterized by ESI-MS and MS/MS.[53–55] Intact phytochelatin–metal complexes have not yet been detected due to their low stability under standard electrospray conditions, nevertheless, ESI-MS and tandem MS studies so far have greatly contributed to unravel the molecular microheterogeneity of plant phytochelatins.

2. Nucleic acids

In the quantitative analysis of covalent nucleic acid adducts with mutagenic agents, a principle problem is the quantification of so far uncharacterized products. This problem has been solved for the quantitative estimation of styrene oxide adducts to nucleotides *via* their analysis by LC-ICP-MS with phosphorus detection.[56] DNA was covalently modified, hydrolyzed and the monomers analyzed by LC-ICP-MS. Covalently modified monomeric units were spotted and quantified by ICP-MS. For these DNA adducts, comparison with parallel investigations by LC-ESI-MS revealed a relatively uniform electrospray ionization efficiency. Adducts of the anticancer drug cisplatin with mononucleotides were also studied by LC-ICP-MS.[57] In this study the covalently modified reaction products could be spotted and quantified by simultaneous detection of phosphorus and platinum. Investigations by ESI-MS were conducted on selected adducts of cisplatin with selected dinucleotides,[58] showing that these intact metal–organic compounds can be detected intact and that the corresponding ESI-MS/MS spectra give insight into their covalent structure.

3. Arsenosugars

Arsenic is occurring in the marine environment and in marine organisms in inorganic form (arsenite/arsenate) and in various organic forms, including methylated arsenic species, such as dimethylarsinic acid, quaternized species such as arsenocholine/arsenobetaine and a variety of arsenoribosides summarized under the term of arsenosugars (*e.g.* ref. 59). In these ribosides, arsenic is located at the position of the 5-hydroxy group of ribose. This class of compounds has been carefully investigated both by LC-ICP, LC-ESI-MS and ESI-MS/MS. With ESI-MS both molecular, structural and sometimes also element information can be obtained, since the arsenosugars give abundant molecular ion signals in ESI, structurally significant fragment ions upon CID include an As^+ fragment signal at *m/z* 75.[60] This fragment ion seems to be absent in normal product ion spectra of arsenosugars,[60] but was observed following collisional activation in the ion transfer region.[61] Both element and molecular ion information by ESI MS was demonstrated also for organotin compounds.[62] These findings highlight the 'dual mode' analytical potential of ESI, provided dedicated instrumental conditions are established. Nevertheless, in the majority of studies on arsenosugars, ICP-MS was used for As detection and quantification. These studies refer to the detection and structural assignment of arsenosugars in kelp,[63] algae,[64,65] and seaweed,[66–69] as well as in urine from sheep[70] and humans[71] following ingestion of arsenosugars. In the application to arsenosugars the analytical strategy has repeatedly proven its capability for detection and structural characterization of novel metabolites. A selenium-containing hexose was also identified by a similar strategy.[72]

4. Hormones and vitamins

Thyroid hormones. Thyroglobulin is the only iodine-containing protein in humans and it contains monoiodo-tyrosine, diiodotyrosine and the thyroid hormones triiodothyronine (T3) and thyroxine (T4). LC-ICP-MS with iodine detection has been used for separation and detection of these iodo amino acids following proteolysis of thyroglobulin.[73] Plasma levels of T3, rT3 (isomeric form of T3), and T4 have been quantified by CE-ICP-MS,[74] including the thyroid hormones in human serum.[75] Joint applications of ESI and ICP-MS in this field are confined to methodological studies,[76] since reference compounds for thyroid hormones are readily available, which enable a reliable identification to be performed by chromatographic retention times. For iodine, ESI-MS can provide element information, *e.g.* by detection of the I^- ion at *m/z* 126.9. However, as is the case for Se and As, the sensitivity for detection of iodine by ICP-MS is substantially higher than that observed for ESI-MS in the 'element mode'.[77]

Cobalt. Cobalt is an essential trace element. By SEC-ICP-MS analysis of liver proteins for Co, a single narrow peak is observed.[57] In low molecular weight fractions cobalt is mainly part of vitamin B_{12} and related substances, the cobalamines. CE-ICP-MS with Co detection has been used for separation of cobalamines and the investigation of vitamin supplements containing vitamin B_{12}.[78] ESI-MS is capable of detecting molecular ion signals for labile molecules such as adenosylcobalamin or cyanocobalamin and was found to be helpful in establishing LC separation conditions for cobalamins free of artifact peaks.[79]

5. Drugs and drug metabolites

Metabolic and pharmacokinetic studies of drugs require sensitive and robust methods, among which ICP-MS is attracting increasing attention, since a variety of drugs contain ICP-detectable elements, such as chlorine, bromine, iodine, sulfur, phosphorus or covalently bound metals. Moreover, unknown compounds may also be quantified by ICP-MS on the basis of such an 'element tag' in case the stoichiometry of its presence can be verified. For this task ESI-MS spectra can provide valuable data. For investigations on the metabolic fate of 4-bromoanaline[80,81] and of a bromo-acetanilide[82] derivative in rats, LC-ICP- and LC-ESI-MS have been successfully used in combination. Also, the metabolic influence of human and rat plasma on brominated bradykinin was studied in an *in vitro* assay by the same methodology.[83] The natural isotopic doublet of ^{79}Br and ^{81}Br is particularly beneficial for the spotting of Br-containing metabolites in the corresponding ESI-MS spectra. Quantification of the chlorine-containing drugs diclofenac and chlorpromazine on the basis of ^{35}Cl detection was shown to be independent of the solvent composition in gradient LC-ICP-MS,[84] and simultaneous detection of ^{35}Cl and ^{32}S allowed the quantitative detection of sulfur-containing metabolites such as a sulfate ester and an *N*-acetyl-cysteinyl conjugate.[85] Sulfur-32 was also demonstrated to be appropriate for the determination of impurities in a drug preparation at a level below 0.1%.[86] The innovative potential of ICP-MS in metallodrug studies was demonstrated in two different ways. (1) In a study of a ^{14}C labelled organoplatinum drug, Pt and ^{14}C were simultaneously monitored by ICP-MS.[87] Monitoring of ^{14}C by its mass instead of its radioactive decay is a new way to gain insight into the integrity of the organic part of the drug. (2) In a study of metallodrug/protein interactions with Ru- and Pt-containing drugs, these elements were monitored in combination with size-exclusion chromatography to study their binding kinetics to

human serum proteins.[88] Finally, first attempts on the quantitative detection of phospholipids by phosphorus-31 detection have been reported within an article summarizing pharmaceutical applications of element mass spectrometry.[89]

Quantitative aspects

The sensitivity of the core analytical techniques reviewed here, LC-MS either in the ICP-MS or ESI-MS mode, is defined by the signal-to-noise ratio of an analyte in its corresponding transient chromatographic peak. The main factors governing the sensitivity are the recovery of the analytes from the LC column, the MS ionization efficiency and the background signal level. Biomolecules show nonspecific absorption on all types of chromatographic materials used for separation in liquid chromatography, an effect which usually is corrected by co-injection of an appropriate internal standard and the recording of calibration curves.

The signal response in ESI-MS is strongly dependent on the chemical nature of the analyte and of the solvent. Due to the pronounced dependency of the signal intensity on analyte and matrix, quantitative results with ESI require the presence of an internal standard for each analyte, such as an isotopically labelled analogue or a homologous compound. The requirements of quantitative measurements by MALDI-MS are similarly strict. Background signals normally are not limiting the sensitivity in the detection of biomolecules by MS, due to the specificity of the relatively high molecular weight of the individual analytes (> 500 Da).

The ionization efficiency in ICP-MS for the metals at one side and semi- or non-metallic elements on the other, varies between one and two orders of magnitude. The variation in corresponding detection limits is much larger. This is mainly caused by the much higher background for some nonmetallic elements, for instance P and S, compared to the extremely low background for some metals, for instance of the lanthanides. Quantitative measurements by LC-ICP-MS require attention mainly with respect to the matrix. In contrast, the chemical nature of individual analytes on the signal intensity is of minor importance, so that usually a single standard can be used as generic reference for a whole class of analytes.

For most applications reviewed here employing both element and molecular mass spectrometry, detection limits are typically in the range of 0.1–10 pmol (eluting from the LC column). Depending on the particular application, either ICP or ESI/MALDI may show more favourable detection limits. For instance, for the detection of lanthanides LC-ICP-MS will provide the lower detection limits, and for the detection of halogenated biomolecules, ESI-MS will probably be more sensitive. As demonstrated in the reviewed studies, the sensitivity ranges of both approaches exhibit a broad overlap allowing their combined application to a palette of problems.

Summary and outlook

In view of the numerous recent articles on the combined application of element and molecular mass spectrometry referenced here one can conclude that an active field of research is identified. The high level of analytical excellence achieved may trigger the development of dedicated instruments to further improve the impact of this combined approach. A simple variant would be a set-up optimized for simultaneous analysis of an LC-eluate by ICP- and ESI-MS. Laser ablation ICP-MS or secondary ion mass spectrometry (SIMS) can provide mass-specific surface images based on element ions (ICP, SIMS) or molecular fragment ions (SIMS), and in fact they are emerging as attractive tools for the characterization of tissue slices or for the read-out of microarrays and of chromatographic or electrophoretic systems. Hybrid element/molecular mass spectrometers, if developed, would offer a significant improvement in analysis speed and probably also in sensitivity over current instrumentation. It appears possible that the traditionally separated development and application of element and molecular mass spectrometry may be overcome in view of the current stimulating results. The implementation of both capabilities into one instrument would fit perfectly to the analytical demands in the life sciences, since the study of the principles and phenomena of life requires an interdisciplinary approach.

Acknowledgements

We are indebted to N. Jakubowski for encouraging support and valuable discussions.

References

1 H. Chassaigne, V. Vacchina and R. Lobinski, *Trends Anal. Chem.*, 2000, 19, 300 313.
2 J. Szpunar and R. Lobinski, *Anal. Bioanal. Chem.*, 2002, 373, 404 411.
3 B. Michalke, *TrAC Trends Anal. Chem.*, 2002, 21, 142 153.
4 B. Michalke, *TrAC Trends Anal. Chem.*, 2002, 21, 154 165.
5 R. Lobinski and J. Szpunar, *Anal. Chim. Acta*, 1999, 400, 321 332.
6 B. Michalke and P. Schramel, *Biol. Trace Element Res.*, 1997, 59, 45 56.
7 M. Kotrebai, M. Birringer, J. F. Tyson, E. Block and P. C. Uden, *Analyst*, 2000, 125, 71 78.
8 S. McSheehy, W. Yang, F. Pannier, J. Szpunar, R. Lobinski, J. Auger and M. Potin-Gautier, *Anal. Chim. Acta*, 2000, 421, 147 153.
9 M. Kotrebai, M. Birringer, J. F. Tyson, E. Block and P. C. Uden, *Anal. Commun.*, 1999, 36, 249 252.
10 C. Casiot, V. Vacchina, H. Chassaigne, J. Szpunar, M. Potin-Gautier and R. Lobinski, *Anal. Commun.*, 1999, 36, 77 80.
11 T. Lindemann and H. Hintelmann, *Anal. Chem.*, 2002, 74, 4602 4610.
12 J. R. Encinar, R. Ruzik, W. Buchmann, J. Tortajada, R. Lobinski and J. Szpunar, *Analyst*, 2003, 128, 220 224.
13 J. R. Encinar, L. Querdane, W. Buchmann, J. Tortajada, R. Lobinski and J. Szpunar, *Anal. Chem.*, 2003, 75, 3765 3774.
14 S. McSheehy, S. Pohl, J. Szpunar, M. Potin-Gautier and R. Lobinski, *J. Anal. At. Spectrom.*, 2001, 16, 68 73.
15 S. McSheehy, F. Pannier, J. Szpunar, M. Potin-Gautier and R. Lobinski, *Analyst*, 2002, 127, 223 229.
16 D. Fagegaltier, A. Lescure, R. Walczak, P. Carbon and A. Krol, *Nucleic Acids Res.*, 2000, 28, 2679 2689.
17 V. Mostert, *Arch. Biochem. Biophys.*, 2000, 376, 433 438.
18 K. Ishida, T. Morino, T. Takagi and Y. Sukenaga, *Nucleic Acids Res.*, 1987, 15, 10051.
19 D. L. St. Germain and V. A. Galton, *Thyroid*, 1997, 7, 655 668.
20 H. Koyama, K. Omura, A. Ejima, Y. Kasanuma, C. Watanabe and H. Satoh, *Anal. Biochem.*, 1999, 267, 84 91.
21 S. Yoneda and K. T. Suzuki, *Biochem. Biophys. Res. Commun.*, 1997, 231, 7 11.
22 U. Sidenius, O. Farver, O. Jons and B. Gammelgaard, *J. Chromatogr., B*, 1999, 735, 85 91.
23 S. Mu, K. E. Hill, R. M. Caprioli and R. F. Burk, *J. Biol. Chem.*, 2002, 277, 12749 12754.
24 S. Ma, R. M. Caprioli, K. E. Hill and R. F. Burk, *J. Am. Soc. Mass Spectrom.*, 2003, 14, 593 600.
25 M. Wind, A. Wegener, R. Kellner and W. D. Lehmann, *Angew. Chem. Int. Ed. Engl.*, 2003, 115, 3425 3427.
26 P. Blume-Jensen and T. Hunter, *Nature*, 2001, 411, 355 365.
27 M. Wind, M. Edler, N. Jakubowski, M. Linscheid, H. Wesch and W. D. Lehmann, *Anal. Chem.*, 2001, 73, 29 35.
28 M. Wind, D. Gosenca, D. Kübler and W. D. Lehmann, *Anal. Biochem.*, 2003.
29 M. Wind, O. Kelm, E. Nigg and W. D. Lehmann, *Proteomics*, 2002, 2, 1516 1523.
30 O. Kelm, M. Wind, W. D. Lehmann and E. A. Nigg, *J. Biol. Chem.*, 2002, 277, 25247 25256.
31 M. Wind, L. Feldmann, N. Jakubowski and W. D. Lehmann, *Electrophoresis*, 2003, 24, 241276 241280.
32 J. S. Becker, S. F. Boulyga, J. S. Becker, C. Pickhardt, E. Dunoc and M. Przybylski, *Int. J. Mass Spectrom.*, 2003, 228, 985 997.

33 M. Wind, H. Wesch and W. D. Lehmann, *Anal. Chem.*, 2001, 73, 3006 3010.
34 D. R. Bandura, V. I. Baranov and S. D. Tanner, *Anal. Chem.*, 2002, 74, 1497 1502.
35 S. D. Tanner, V. I. Baranov and D. R. Bandura, *Spectrochim. Acta, B*, 2002, 57, 1361 1452.
36 S. E. O'Brien, B. W. Acon, S. F. Boulyga, J. S. Becker, H. J. Dietze and A. Montaser, *J. Anal. At. Spectrom.*, 2003, 18, 230 238.
37 J. Wang, D. Dreessen, D. R. Wiederin and R. S. Houk, *Anal. Biochem.*, 2001, 288, 89 96.
38 I. Leopold and B. Fricke, *Anal. Biochem.*, 1997, 252, 277 285.
39 A. Leber, B. Hemmens, B. Klösch, W. Goessler, G. Raber, B. Mayer and K. Schmidt, *J. Biol. Chem.*, 1999, 274, 37658 37664.
40 B. Hemmens, W. Goessler, K. Schmidt and B. Mayer, *J. Biol. Chem.*, 2000, 275, 35786 35791.
41 R. Lobinski, H. Chassaigne and J. Szpunar, *Talanta*, 1998, 46, 271 289.
42 S. McSheehy and Z. Mester, *TrAC Trends Anal. Chem.*, 2003, 22, 311 326.
43 H. Chassaigne and R. Lobinski, *J. Chromatogr., A*, 1998, 829, 127 136.
44 K. Polec, O. Garcia-Arribas, M. Perez-Calvo, J. Szpunar, B. Ribas-Ozonas and R. Lobinski, *J. Anal. At. Spectrom.*, 2000, 15, 1363 1368.
45 K. Polec, M. Perez-Calvo, O. Garcia-Arribas, J. Szpunar, B. Ribas-Ozonas and R. Lobinski, *J. Inorg. Biochem.*, 2002, 88, 197 206.
46 A. N. Richarz and P. Brätter, *Anal. Bioanal. Chem.*, 2002, 372, 412 417.
47 A. Prange and D. Schaumlöffel, *Anal. Bioanal. Chem.*, 2002, 373, 441 453.
48 C. Alfonso, Y. Hathout and C. Fenselau, *J. Mass Spectrom.*, 2002, 37, 755 759.
49 D. Schaumlöffel, A. Prange, G. Marx, K. G. Heumann and P. Brätter, *Anal. Bioanal. Chem.*, 2002, 372, 155 163.
50 Z. Wang and A. Prange, *Anal. Chem.*, 2002, 74, 626 631.
51 C. Zhang, Z. Zhang, B. Yu, J. Shi and X. Zhang, *Anal. Chem.*, 2002, 74, 96 99.
52 V. I. Baranov, Z. Quinn, D. R. Bandura and S. D. Tanner, *Anal. Chem.*, 2002, 74, 1629 1636.
53 V. Vacchina, R. Lobinski, M. Oven and M. H. Zenk, *J. Anal. At. Spectrom.*, 2000, 15, 529 534.
54 V. Vacchina, H. Chassaigne, M. Oven, M. H. Zenk and R. Lobinski, *Analyst*, 1999, 124, 1425 1430.
55 H. Chassaigne, V. Vacchinas, T. M. Kutchan and M. H. Zenk, *Phytochemistry.*, 2001, 56, 657 668.
56 C. Siethoff, C. I. Feldmann, N. Jakubowski and M. Linscheid, *J. Mass Spectrom.*, 1999, 34, 421 426.
57 S. Hann, A. Zenker, M. Galanski, T. L. Bereuter, G. Stingeder and B. K. Keppler, *Fresenius' J. Anal. Chem.*, 2000, 370, 581 586.
58 T. Hagemeister and M. Linscheid, *J. Mass Spectrom.*, 2002, 37, 731 747.
59 J. J. Corr and E. H. Larsen, *J. Anal. At. Spectrom.*, 1996, 11, 1215 1224.
60 S. A. Pergantis, S. Wangkarn, K. A. Francesconi and J. E. Thomas-Oates, *Anal. Chem.*, 2000, 72, 357 366.
61 D. Kuehnelt, W. Goessler and K. A. Francesconi, *Rapid Commun. Mass Spectrom.*, 2003, 17, 654 659.
62 J. J. Corr, *J. Anal. At. Spectrom.*, 1997, 12, 537 546.
63 P. A. Gallagher, X. Wei, J. A. Shoemaker, C. A. Brockhoff and J. T. Creed, *J. Anal. At. Spectrom.*, 1999, 14, 1829 1834.
64 A. D. Madsen, W. Goessler, S. N. Pedersen and K. A. Francesconi, *J. Anal. At. Spectrom.*, 2000, 15, 657 662.
65 S. McSheehy, P. Pohl, D. Velez and J. Szpunar, *Anal. Bioanal. Chem.*, 2002, 372, 457 466.
66 S. McSheehy, M. Marcinek, H. Chassaigne and J. Szpunar, *Anal. Chim. Acta*, 2000, 410, 71 84.
67 P. A. Gallagher, J. A. Shoemaker, X. Wei, C. A. Brockhoff-Schwegel and J. T. Creed, *Fresenius' J. Anal. Chem.*, 2001, 369, 71 80.
68 M. Miguens-Rodriguez, R. Pickford, J. E. Thomas-Oates and S. A. Pergantis, *Rapid Commun. Mass Spectrom.*, 2002, 16, 323 331.
69 M. Van Hulle, C. Zhang, X. Zhang and R. Cornelis, *Analyst*, 2002, 127, 634 640.
70 K. A. Francesconi, R. Tanggard, C. J. McKenzie and W. Goessler, *Clin. Chem.*, 2002, 48, 92 101.
71 H. R. Hansen, A. Raab and J. Feldmann, *J. Anal. At. Spectrom.*, 2003, 18, 474 479.
72 Y. Ogra, K. Ishiwata, H. Takayama, N. Aimi and K. T. Suzuki, *J. Chromatogr., B*, 2002, 767, 301 312.
73 B. Michalke and P. Schramel, *Electrophoresis*, 1999, 20, 2547 2553.
74 K. Takatera and T. Watanabe, *Anal. Chem.*, 1993, 65, 759 762.
75 B. Michalke, P. Schramel and P. Witte, *Biol. Trace Element Res.*, 2000, 78, 81 91.
76 M. Wind, A. Eisenmenger and W. D. Lehmann, *J. Anal. At. Spectrom.*, 2002, 17, 21 26.
77 W. Buchberger, B. Crizsek, S. Hann and G. Stingeder, *J. Anal. At. Spectrom.*, 2003, 18, 512 514.
78 S. A. Baker and N. J. Miller-Ihli, *Spectrochim. Acta, B*, 2000, 55, 1823 1832.
79 H. Chassaigne and R. Lobinski, *Anal. Chim. Acta*, 1998, 359, 227 235.
80 J. K. Nicholson, J. C. Lindon, G. Scarfe, I. D. Wilson, F. Abou-Shakra, J. Castro-Perez, A. Eaton and S. Preece, *Analyst*, 2000, 125, 235 236.
81 F. R. Abou-Shakra, A. B. Sage, J. Castro-Perez, J. C. Lindon, G. B. Scarfe and I. D. Wilson, *Chromatographia Suppl.*, 2002, 55, S-9 S-13.
82 J. K. Nicholson, J. C. Lindon, G. B. Scarfe, I. D. Wilson, F. Abou-Shakra, A. B. Sage and J. Castro-Perez, *Anal. Chem.*, 2001, 73, 1491 1494.
83 P. Marshall, O. Heudi, S. Mckeown, A. Amour and F. Abou-Shakra, *Rapid Commun. Mass Spectrom.*, 2002, 16, 220 228.
84 C. J. Duckett, N. J. C. Bailey, H. Walker, F. Abou-Shakra, I. D. Wilson, J. C. Lindon and J. K. Nicholson, *Rapid Commun. Mass Spectrom.*, 2002, 16, 245 247.
85 O. Corcoran, J. K. Nicholson, E. M. Lenz, F. Abou-Shakra, J. Castro-Perez, A. B. Sage and I. D. Wilson, *Rapid Commun. Mass Spectrom.*, 2000, 14, 2377 2384.
86 E. H. Evans, J. C. Wolff and C. Eckers, *Anal. Chem.*, 2001, 73, 4722 4728.
87 C. J. Smith, I. D. Wilson, F. Abou-Shakra, R. Payne, H. Griesdale, A. Long, D. Roberts and M. Malone, *Chromatographia Suppl.*, 2002, 55, S-151 S-155.
88 J. Szpunar, A. Makarov, T. Pieper, B. K. Keppler and R. Lobinski, *Anal. Chim. Acta*, 1999, 387, 135 144.
89 B. O. Axelsson, M. Joernten-Karlsson, P. Michelsen and F. Abou-Shakra, *Rapid Commun. Mass Spectrom.*, 2001, 15, 375 385.